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Intake pattern and nutrient supply of lactating sheep selecting dried forage from woody plants and straw offered in binary or multiple choice

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Intake pattern and nutrient supply of lactating sheep selecting dried forage from woody plants and straw offered in binary or multiple choice

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ABSTRACT

This study investigated whether offering either binary or multiple choice among low quality forages has an effect on intake and feeding behaviour compared to no-choice situations where only one single low-quality forage is offered. Forages from different woody plants and straw were tested in lactating sheep either in Syria (Exp. 1; Awassi sheep) or in Switzerland (Exp. 2; East Friesian Dairy sheep). *Artemisia herba-alba*, *Atriplex leucoclada*, *Haloxylon articulatum*, *Noaea mucronata*, and *Salsola vermiculata* were used in Exp. 1. The three most preferred plants (*A. leucoclada*, *H. articulatum* and *S. vermiculata*) were tested again in Exp. 2 together with *Betula pendula*, *Castanea sativa* and *Juglans regia*. Both experiments started with a binary choice test where one plant and barley straw were offered separately for 4 h in the morning to six sheep (test period) in a random sequence in consecutive 7-day subperiods. A control group ($n = 6$ per experiment) received only straw in the test period. For the rest of the day, a basal diet composed of straw *ad libitum* and concentrate was offered. After the binary choice test, two 2-week periods followed, where in the first straw only and in the second all test plants were offered to all animals from both groups to ascertain equal familiarisation with all plants. For the following 7-day multiple choice test animals were allocated to two new groups. The 'multiple choice' group could choose among all test plants and straw during the 4-h test period, the 'control' group received only the basal diet. Intakes of test feeds during 4 h and 24 h as well as feeding behaviour during the first 30 min were recorded. Additionally, nutrient intake was determined. Total daily test feed intake was always higher in the choice groups, but this was more pronounced in the multiple choice situation (Exp. 1: 30 and 48, Exp. 2: 49 and 74 g dry matter/kg live-weight$^{0.75}$ with 'control' and 'multiple choice', respectively). *A. leucoclada* (Exp. 1; proportionately 0.73 of total test plant intake) and *B. pendula* (Exp. 2; 0.87) were the preferred plants in the multiple choice test and also with binary choice. Most other feeds were only consumed in low amounts in the binary and even less in multiple choice situations. In conclusion, giving sheep the choice among low quality forages seemed to be advantageous. Even though in both experiments animals preferred especially one plant, choice still facilitated intake.

Abbreviations: ADFom, ash-free acid detergent fibre; aNDFom, ash-free neutral detergent fibre; CP, crude protein; CT, condensed tannins; DM, dry matter; EE, ether extract; Exp., experiment; lignin(sa), lignin determined by solubilisation of cellulose with sulphuric acid; non-fibre CHO, non-aNDFom carbohydrates; OM, organic matter; TP, total phenols; TT, total tannins.

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1. Introduction

In the Mediterranean area, livelihood of nomadic and semi-nomadic people is based on rangelands which are the major feed source for their animals (Louhaichi and Tastad, 2010). However, dry and semi-arid areas are affected by climate change manifested with more pronounced droughts and more irregular precipitation events (Hadri and Guellouz, 2011) which together with overgrazing facilitate degradation of the rangelands (Louhaichi and Tastad, 2010). The latter group of authors stated that, therefore, farmers are shifting to systems consisting of a combination of free-range grazing and supplementary feeding and local governments initiated re-cultivation programmes with woody plants to stop and reverse degradation of the rangelands. The use of woody plants as supplements during the dry season might become increasingly important. In arid areas several woody plants are used as potentially high quality supplements to the available low protein forage (Leng, 1997). However, only few woody plant species were evaluated for their acceptability by sheep. The feeding value of woody plants might be impaired as they may contain substantial levels of potentially harmful compounds. An increasing plant species diversity of the diet gives the animals the opportunity to compose their own mixed diet (Duncan et al., 2003). This enables the animal to consume more plants either complementary in nutrient composition, or with potentially harmful compounds (Lyman et al., 2008) by benefitting from the biochemical complementarity of these compounds (Manteca et al., 2008). Generally, a more diverse feed offer could increase intake (Rogosic et al., 2008) by increasing the animals' motivation to eat (Ginane et al., 2002) and improve performance by giving the animals the chance to select a nutritionally optimised diet (Duncan et al., 2003).

The hypotheses tested in the present study were that (i) offering different low quality forages in a binary or a multiple choice situation leads to a higher total intake during the choice feeding period and across the entire day, and (ii) a choice feeding situation improves nutrient intake compared to no-choice situations. Differences in preference pattern were expected with regard to the different woody plants on offer; however, no difference in preference pattern between the binary and the multiple choice situation was anticipated. For this purpose, two experiments were conducted in different climatic regions with two local dairy sheep breeds testing various woody forages and straw.

2. Materials and methods

2.1. Study regions

The sites selected were located in the Mediterranean region, having a warm and dry climate, and in central Europe, with a temperate and more humid climate. The Mediterranean experiment (Exp. 1) was conducted at the International Center for Agricultural Research in the Dry Areas (ICARDA, sheep barn: 36.0245 N, 36.9545 E, 296 m a.s.l.), Aleppo, Syria, from April to July 2010 (spring and summer). The central European experiment (Exp. 2) was conducted at the Institute of Animal Nutrition of the University of Zurich, Zurich, Switzerland (sheep barn: 47.3946 N, 8.5507 E, 518 m a.s.l.), from June to September 2011 (summer). The procedures followed in Exp. 2 were approved by the Cantonal Veterinary Office of Zurich. Exp. 1 was identical in terms of animal handling; however, in Syria no approval procedures were established by the government. The design of the two experiments was in agreement with the "International Guiding Principles for Biomedical Research Involving Animals" issued by the Council for International Organizations of Medical Sciences.

2.2. Test feeds and basal diet

For Exp. 1, leaves with twigs of five different sprouting steppe shrub species (*Artemisia herba-alba*, *Atriplex leucoclada*, *Haloxylon articulatum*, *Noaea mucronata*, and *Salsola vermiculata*) were collected in the Syrian steppe in March and April 2010. The plant material was air-dried in the shade for several days before it was offered as test plants to the sheep. For Exp. 2, dried material from the three best eaten shrubs from Exp. 1 (*A. leucoclada*, *H. articulatum*, and *S. vermiculata*) was chopped to a maximal size of the twigs and leaves of about 3 and 1 cm, respectively, and then transported to Switzerland. Additionally, leaves of the tree species *Betula pendula*, *Castanea sativa* and *Juglans regia* were purchased from Alfred Galke GmbH (Gilltelde, Germany). These leaves had been harvested in Albania (*B. pendula*, *C. sativa*) and in Bulgaria (*J. regia*), dried in the shade and chopped to a maximum size of 1 cm before selling. In both experiments local chopped barley straw was used as low quality control forage.

The basal diet which was offered daily during the entire experiments to meet the animals' requirements for maintenance and lactation consisted of barley straw *ad libitum* and concentrate (1070 and, on average, 782 g/day per animal in Exp. 1 and 2, respectively). In Exp. 1 the concentrate was mixed (g/kg) using whole barley grains (882), soybean meal (98), dicalcium phosphate (12), sodium chloride (6), and vitamin–mineral premix (2). The latter provided per kg of concentrate: Ca, 270 mg; Na, 243 mg; P, 212 mg; Mn, 1.52 mg; Mg, 0.30 mg; Co, 0.23 mg; I, 0.15 mg; vitamin A, 24,500 IU; vitamin D_3, 600 IU; vitamin E, 1 mg. In Exp. 2, a concentrate designated for dairy sheep and goats (Combifloc 2957C) was purchased from a commercial producer (Melior AG, Herzogenbuchsee, Switzerland). It consisted of wheat, barley, wheat starch, wheat and maize flakes, various mill by-products, distiller's grains, soybean meal, maize gluten, canola expeller, sugar beet pulp, molasses, and vegetable oil. It also included a mineral-vitamin premix in an amount providing, per kg of concentrate, Ca, 11 g; P, 5.5 g; Mg, 3 g; vitamin A, 25,000 IU; vitamin D_3, 5000 IU, vitamin E, 25 mg. Additionally the sheep of Exp. 2 were given continuous

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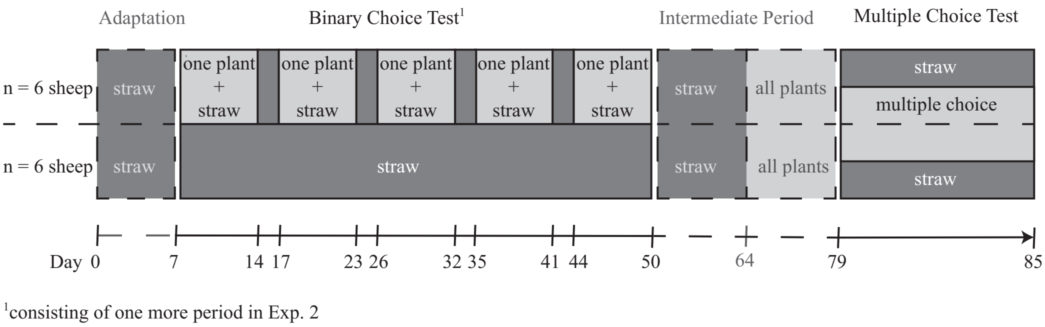

Fig. 1. Design of the experiments.

access to a mineral lick (AgroLine AG, Roggwil, Switzerland) which provided per kg: Na, 375 g; Mg, 8 g; Zn, 800 mg; Mn, 80 mg; I, 100 mg; Se, 30 mg; Co, 30 mg.

2.3. *Animals and housing*

In Exp. 1, 12 lactating fat-tailed Awassi sheep (weighing 60.2 ± 7.24 kg) were selected for the experiment. In Exp. 2, 12 lactating East Friesian Dairy sheep (64.0 ± 12.57 kg) were used. The experiments started when the sheep had passed their 8th week of lactation. They had no previous experience with the test feeds offered (except with barley straw in Exp. 1) and had been kept in their respective herds before starting with the experiments. The animals in Exp. 1 grazed local pasture and were supplemented with grass silage during grazing and concentrate during milking prior to the experiments. The animals of Exp. 2 had been kept indoors and were fed with grass hay and concentrate prior to the experiment. One week before starting the experiments the animals were penned individually in barns. The pens had sizes of 2 m × 2 m in Exp. 1 and 1.2 m × 2 m in Exp. 2. Water was available at *ad libitum* access. In order to prevent litter intake, the animals were kept on bare concrete floor (Exp. 1) or on wood shavings (Exp. 2). All animals were weighed at the beginning of each period/sub-period before morning feeding time, but without previous feed or water restriction (measurements per animal were used for calculations of feed intake per animal, one measurement per period/sub-period), and were milked by hand twice a day. Every morning after milking the animals received the test feeds (woody test plants or straw or both) during 4 h (further on called 'test period'). The troughs were filled when the animals were standing in the opposite corner of their pens (Exp. 1) or when they were staying in the milking parlour (Exp. 2). This approach ensured that the animals were confronted with the entire feed choice and did not start eating the feed that was provided first. During the test period the troughs were refilled if needed to allow always an *ad libitum* intake. The animals remained undisturbed for 30 min when the troughs were first refilled. After 4 h the test feeds were withdrawn and the animals received the basal diet for the rest of the day.

2.4. *Design of the experiments*

Both experiments followed the same protocol taking into account important issues which could influence choice experiments (Meier et al., 2012). Before starting the experiments, the animals passed a 7-day adaptation period (Fig. 1) to get familiar with the individual pens, feeding and other housing management aspects like it was done by Görgülü et al. (1996). During that time no test plants were offered, but all troughs used later in the experiment were already present to familiarise the animal with the set-up. At the end of this week, the animals showed no signs of stress and a constant feed intake. Afterwards a binary choice test started which was divided into five (Exp. 1) or six (Exp. 2) sub-periods of 7 days each. Half of the animals each were allocated either to a 'binary choice' group or a 'control' group. The 'binary choice' group was offered one of the test plants (in a randomised order between animals) and barley straw in separate troughs during the test period while the control group received only barley straw. The allocation of the feeds to the troughs was changed daily in both groups to prevent a 'habit reflex' (Kaitho et al., 1996). In order to minimise carry-over effects of the previous choice situation, 2 days followed between sub-periods where only barley straw but no test plant was offered. This schedule was repeated until each plant had been offered to each animal from the 'binary choice' group once for 7 days. In Exp. 2, one control animal had to be removed in the last sub-period due to illness (repeated bloating followed by a severe decrease of intake) for the rest of the experiment. In order to minimise any group differences resulting from the binary choice test, 14 days of offering only straw and 14 days of offering all plants during the daily test periods in order to familiarise all animals with every test plant (intermediate period, Fig. 1) were inserted. In that period, the effects of previous experience on test plant intake were tested. The results of this comparison are described elsewhere (Meier et al., 2013). For the actual multiple choice test, the animals were then allocated to two new groups each consisting of three animals of the former binary choice and three of the former control group. The 'multiple choice' group, was given simultaneous access to all test plants and straw in separate troughs during the test period. The new 'control' group (only $n = 5$ in Exp. 2) received only straw in the test period. The multiple choice test lasted for 7 days. Test feed position was changed daily.

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2.5. Measurement of feeding pattern

Dry matter intake per feed was measured in the test period (4 h) and during 24 h by recording supply and refusals in the binary and multiple choice test. In Exp. 1, the 4-h straw intake of the control animals during the test period was not recorded in the binary choice test. Total test plant intake (4 h) and total test feed intake (24 h) was calculated by summing up the intake of the corresponding feeds. All absolute intake data are given in relation to metabolic live-weight (live-weight$^{0.75}$) to balance individual weight differences. This relationship was chosen as covering maintenance energy requirements, which depend on metabolic weight, was a major motivation for feed intake in the animals with their limited milk yield. Feeding behaviour was recorded by four video cameras (CANON Legria FS200, Tokyo, Japan) during the first 30 min of the test periods. In Exp. 2, due to space restrictions only 5 of the 6 choice fed animals in the binary choice test could be housed in the same part of the building and observed with respect to their feeding behaviour. The time periods of eating from the individual feeds (starting when the animal moved its head into a trough and showed chewing movements which indicated eating, and ending when it moved its head away) and switching between different feeds (counts of visits to the individual feeding troughs) were analysed only for the choice groups using the behaviour software INTERACT (Mangold International GmbH, Arnstorf, Germany).

2.6. Laboratory analyses

Feed samples (n = 8 and 4 per feed in Exp. 1 and 2, respectively) were analysed in the local laboratories after grinding to pass a 0.75 mm sieve. In Exp. 1, samples were analysed as outlined by AOAC (1997) for dry matter (DM) and organic matter (OM; AOAC No. 930.15). In Exp. 2, a TGA-701 (Leco Corporation, St. Joseph, MI, USA) was used for DM and ash analysis. Detergent fibre analysis was made according to Goering and van Soest (1970), and AOAC (No. 973.18) by using the Fibertec System M (Tecator, 1020 Hot Extraction, Flawil, Switzerland) in Exp. 2. For the analysis of neutral detergent fibre, α-amylase was used but no sodium sulphite (van Soest et al., 1991). The neutral and acid detergent fibre values are always given exclusive of ash (aNDFom and ADFom) and sulphuric acid was used for analysing lignin (lignin(sa)). In Exp. 1, crude protein (CP) analysis was done by the Kjeldahl method (AOAC No. 991.20) using a digestion block heater (Tector 2020, Foss, Hillerød, Denmark) and a distillation unit (Vapodest30, Gerhardt GmbH, Königswinter, Germany). In Exp. 2, nitrogen contents were determined using a C/N-Analyser (Leco-Analyser Typ FP-2000, Leco Corporation, St. Joseph, MI, USA; AOAC No. 977.02). Crude protein was calculated as 6.25 × N. Ether extract (EE) was analysed with a Soxhlet extractor (in Exp. 1: Soytherms306 A, Gerhardt GmbH, Königswinter, Germany; AOAC No. 920.39; in Exp. 2: Extraktionssystem B-811, Büchi, Flawil, Switzerland; AOAC No. 963.15). Total tannins (TT) and total phenols (TP) were determined in all samples according to the Folin–Ciocalteau method (Makkar, 2003) with slight modifications using gallic acid as standard. Condensed tannins (CT) were analysed according to the butanol–HCl–iron method and the values are given as leucocyanidin equivalents. Furthermore, contents of Na, K, Ca, and Mg were determined (AOAC No. 980.03, 2000). The contents of non-fibre carbohydrates (non-fibre-CHO) were calculated by the subtraction of aNDFom, CP, EE and TP from OM.

2.7. Statistical analysis

The statistical analysis was done with the MIXED procedure of SAS 9.3 (SAS, 2011). Model 1 was used for the data of the binary and multiple choice tests in order to analyse the effects of the group (choice *vs.* control) and Model 2 to analyse the effects of the test feeds (choice group only):

$$Y_{ijk} = G_i + D_j + G_i \times D_j + e_{ijk} \quad (1)$$

$$Y_{ijk} = F_i + D_j + F_i \times D_j + e_{ijk} \quad (2)$$

The response variables (Y) in Model 1 were total test feed intake (4 h and 24 h), total test plant intake (4 h) and straw intake (4 h and 24 h) and in Model 2 additionally feeding time and counts of visits. The fixed effects were G (feeding group, further on named "group") in Model 1 and F (forage type, further on named "feed") in Model 2, respectively. The models also included a repeated factor (D, day), with the subject animal nested within group in Model 1 and animal in Model 2. For the analysis of the binary choice test a random factor (S, sub-period) was additionally included. In the binary test of Exp. 1, straw (4 h) and total intake (4 h) were assessed for the choice group only thus no statistical analysis was performed. Some data were not normally distributed according to graphical tests with residual plot and QQ plot of the residuals. Therefore logarithmic transformations were made for the data assessed in the binary choice test of Exp. 1 on test feed intake (4 h), straw intake (4 h), total test feed intake (4 h), as well as on feeding behaviour measured on the test plants. Square root transformations were applied for feeding behaviour on straw. In the binary choice test of Exp. 2, data on visits on test plants and straw were logarithmically transformed and data of feeding time on test plants were square root transformed. In the multiple choice test logarithmic transformation was applied for visits to test feeds (Exp. 1) and square root transformation for visits to test feeds in Exp. 2.

Table 1
Chemical composition[a] of the test feeds (g/kg DM) (on average $n=8/11$ for the Mediterranean plants used in Exp. 1 only/in Exp. 1 and 2, and $n=4$ for temperate climate plants).

Feed	Exp.	DM	OM	aNDFom	ADFom	Lignin(sa)	Non-fibre CHO	CP	EE	Ca	Mg	Na	K	TP[b]	TT[b]	CT[c]
Mediterranean plants																
Artemisia herba-alba	1	929	832	420	348	105	241	125	21.9	45.2	22.8	10.5	17.0	24.5	15.9	0.0
Atriplex leucoclada	1/2	914	803	427	256	65	175	182	12.5	33.4	43.8	29.9	23.2	6.8	4.2	0.1
Haloxylon articulatum	1/2	927	837	458	272	69	166	180	9.7	57.0	46.1	12.1	20.6	22.9	13.2	0.3
Noaea mucronata	1	931	873	659	453	124	122	80	6.7	53.5	22.5	6.9	12.5	5.5	2.8	0.1
Salsola vermiculata	1/2	915	871	634	378	103	111	114	7.5	36.8	32.1	18.2	13.7	5.1	2.8	0.2
Temperate climate plants																
Betula pendula	2	916	952	494	295	193	226	155	45.3	n.d.[d]	n.d.	n.d.	n.d.	30.4	24.0	18.3
Castanea sativa	2	917	947	431	272	113	287	120	62.6	n.d.	n.d.	n.d.	n.d.	47.0	41.0	4.9
Juglans regia	2	908	881	477	345	161	201	137	32.6	n.d.	n.d.	n.d.	n.d.	34.4	28.3	12.1
Barley straw																
	1	932	869	669	417	42	139	54	12.0	n.d.	n.d.	n.d.	n.d.	7.7	4.3	0.1
	2	920	926	809	514	81	58	41	12.2	n.d.	n.d.	n.d.	n.d.	5.5	3.1	0.2
Concentrate																
	1	920	918	340	111	7	n.d.	170	16.8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	2	903	912	220	81	33	370	277	41.4	n.d.	n.d.	n.d.	n.d.	3.7	1.4	0.2

[a] CP, crude protein; DM, dry matter, EE, ether extract; non-fibre CHO, non-aNDFom carbohydrates, calculated as OM-aNDFom-CP-EE-TP; OM, organic matter; TP, total phenols; TT, total tannins; CT, condensed tannins.
[b] Given as gallic acid equivalents ($n=4$).
[c] Given as leucocyanidin equivalents ($n=4$).
[d] Not determined.

Model 3 was used to analyse the effect of group in the binary and multiple choice test on average nutrient intake. Model 3 reads:

$$Y_{ijk} = G_i + A(G)_j + e_{ijk} \tag{3}$$

where the fixed effect was G (group). The random effects were in Model 3 animal nested within group ($A(G)$) when analysing data of the multiple choice test and $A(G)$ and S (sub-period) when analysing data of the binary choice test.

Tables and figures give Least Square means by group, day and the interaction thereof (Model 1), by feed, day and the interaction thereof (Model 2) and by group (Model 3). The P-values and the standard errors of the means (SEM) for the fixed effects are shown in the tables only. Multiple comparisons among means were performed using Tukey's test and differences were considered significant at $P<0.05$.

3. Results

3.1. Plant chemical composition

All Mediterranean test plants (*A. herba-alba*, *A. leucoclada*, *H. articulatum*, *N. mucronata*, *S. vermiculata*) contained between approx. 800 and 870 g OM/kg DM (Table 1). *A. leucoclada* had comparably high contents of ash, Na and K as compared to the other plants. Additionally, *A. leucoclada* had a high CP content, together with *H. articulatum*. Most test plants had moderate fibre contents (400–500 g aNDFom/kg DM) and quite substantial contents of non-fibre-CHO. Only *N. mucronata* and *S. vermiculata* approached the high fibre and low non-fibre-CHO contents of the straw. Concerning lignin(sa), most of the woody plants exceeded the level of the two straws used. The straw used in Exp. 1 was of a numerically better quality as that available for Exp. 2. *B. pendula*, *J. regia* and *N. mucronata* were most lignified while *C. sativa* had a high content of TP and TT. In general the dried plant material of the temperate climate plants had numerically higher contents of phenolic compounds compared to the Mediterranean plants.

3.2. Feeding pattern in the binary choice situation

Across all test plants, total test feed intake during the test period (4 h) was higher ($P<0.001$) for the binary choice group than for the control group in Exp. 2 (Table 2; no corresponding data for Exp. 1 available). Additionally, in both experiments a higher ($P=0.03$ and $P=0.002$, in Exp. 1 and Exp. 2, respectively) total test feed intake during 24 h was found for the binary choice group. Offering the animals a binary choice instead of only straw during 4 h enhanced ($P<0.05$) CP intake from test feeds in both experiments (Table 3). Still the concentrate provided the largest part of CP. Non-fibre-CHO intake from test feeds only was higher ($P=0.007$ and $P=0.005$, in Exp. 1 and Exp. 2, respectively) in the binary choice group in both experiments. The higher total daily test feed intake with binary choice was associated with a higher ($P=0.020$ in Exp. 1 and $P=0.005$ in Exp. 2) lignin(sa) intake. In Exp. 2 the binary choice group ingested more ($P=0.007$) TP than the control group when only nutrients from test feeds were considered. For the ingestion of fibre no group differences were found in Exp. 1 and only trends for a higher intake in Exp. 2 ($P=0.05$ and $P=0.06$ for aNDFom and ADFom from test feeds, respectively).

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Table 2
Intake (g dry matter/kg live-weight$^{0.75}$) of plants, straw and total test feed (plants and straw together) when offered in binary and multiple choice situations on average of the 7 day periods.

		Binary choice					Multiple choice				
		Within 4 h			Within 24 h		Within 4 h			Within 24 h	
		Plants	Straw	Total	Straw	Total	Plants	Straw	Total	Straw	Total
Experiment 1											
Control		–	n.d.	n.d.	34.4	34.4	–	13.3	13.3	30.0	30.0
Choice		3.7	11.4	15.1	34.5	38.2	14.9	8.8	23.6	33.0	47.9
Group:	SEM	0.80	1.13	0.99	1.40	1.37	1.48	1.25	1.41	1.53	1.69
	P-value	–	–	–	0.97	0.029	–	0.027	<0.001	0.19	<0.001
Day:	SEM	1.13	1.26	1.39	1.18	1.21	1.72	1.46	1.59	1.34	1.58
	P-value	0.86	0.49	0.88	0.90	0.95	0.006	0.003	0.27	0.30	0.17
Group × Day:	SEM	–	–	–	1.57	1.60	–	2.06	2.25	1.89	2.23
	P-value	–	–	–	0.71	0.82	–	0.82	0.20	0.034	0.007
Experiment 2											
Control		–	18.0	18.0	41.7	41.7	–	17.7	17.7	48.7	48.7
Choice		8.3	17.0	25.3	45.1	53.4	34.4	6.9	41.3	39.4	73.8
Group:	SEM	2.12	1.69	1.63	3.08	2.93	6.60	1.68	4.48	3.24	5.26
	P-value	–	0.64	<0.001	0.42	0.002	–	0.001	0.005	0.075	0.008
Day:	SEM	2.75	1.40	1.53	2.41	2.38	6.83	1.55	3.47	2.53	4.00
	P-value	0.95	0.24	0.91	0.98	0.96	0.072	<0.001	<0.001	<0.001	0.025
Group × Day:	SEM	–	1.88	2.05	3.28	3.17	–	3.41	4.88	3.41	5.63
	P-value	–	0.088	0.48	0.88	0.53	–	0.032	0.76	0.45	0.62

–, not available; n.d., not determined; SEM, standard error of the means.

Within the binary choice groups, the 4-h intakes measured for individual plants and total test feeds differed in both experiments between test plants offered (P<0.001 in both experiments, Table 4). Straw intake in the test period was affected (P<0.001) by the respective test plant offered in the binary choice combinations in Exp. 2, but not in Exp. 1 (P=0.17; Table 4). However, 24-h straw intake differed (P=0.002 and P<0.001, in Exp. 1 and Exp. 2, respectively) between the different binary combinations in both experiments, being lowest when offered together with the respective most consumed test plant. Straw intake during 4 h was always higher than test plant intake, except with *A. leucoclada* in Exp. 1 where the difference was not significant and with *B. pendula* in Exp. 2 where plant intake compared to straw intake during 4 h was higher (Table 4). In Exp. 1, *A. leucoclada* and *S. vermiculata* were the most consumed plants (P<0.05 relative to the others). This resulted in the highest (P<0.05) total test feed intakes during 4 h and 24 h for the binary choice with *A. leucoclada* (P<0.05), followed by the binary choice with *S. vermiculata*. Intake of *A. leucoclada* comprised of almost 240 g/kg of the total daily test feed intake. In Exp. 2, *B. pendula* was the most consumed test plant (about 390 g/kg of total 24-h test feed intake, respectively) followed by *J. regia* (210 g/kg), also resulting in the highest total test feed intakes (24 h) as compared to the other binary choice combinations. Intakes of individual test plants did not differ between days (Table 4 and Fig. 2.1b, 2.2b).

Table 3
Effect of offering binary and multiple choice among feeds on total nutrient intake (g/kg live-weight$^{0.75}$) and nutrient intake from test feeds exclusive of the concentrate (values in brackets).

	Binary choice				Multiple choice			
	Choice	Control	SEM	P-value	Choice	Control	SEM	P-value
Experiment 1								
OM	75.7 (32.8)	73.9 (29.8)	1.67 (1.07)	0.46 (0.080)	84.7 (40.3)	70.0 (25.7)	2.56 (1.43)	0.002 (<0.001)
CP	11.6 (2.5)	11.2 (1.9)	0.33 (0.17)	0.45 (0.004)	11.9 (4.8)	9.1 (2.0)	0.38 (0.17)	<0.001 (<0.001)
aNDFom	38.8 (24.4)	37.7 (22.8)	0.87 (0.83)	0.38 (0.23)	44.1 (26.2)	36.3 (18.4)	1.35 (0.99)	0.002 (<0.001)
ADFom	20.9 (15.1)	20.2 (14.2)	0.51 (0.52)	0.32 (0.25)	21.3 (16.6)	16.3 (11.6)	0.70 (0.62)	<0.001 (<0.001)
Lignin(sa)	2.2 (1.6)	2.0 (1.4)	0.23 (0.23)	0.022 (0.020)	2.3 (2.2)	1.2 (1.1)	0.10 (0.09)	<0.001 (<0.001)
Non-fibre CHO[a]	n.d. (5.1)	n.d. (4.3)	– (0.20)	– (0.007)	n.d. (8.4)	n.d. (4.7)	– (0.28)	– (<0.001)
EE	1.3 (0.5)	1.3 (0.5)	0.08 (0.07)	0.55 (0.073)	1.3 (0.6)	1.1 (0.3)	0.04 (0.02)	0.002 (<0.001)
Total phenols	n.d. (0.3)	n.d. (0.3)	– (0.01)	– (0.073)	n.d. (0.4)	n.d. (0.2)	– (0.02)	– (<0.001)
Experiment 2								
OM	81.8 (49.2)	75.9 (38.3)	4.66 (2.78)	0.39 (0.016)	93.0 (68.6)	72.5 (45.1)	5.94 (4.86)	0.039 (0.008)
CP	13.0 (2.8)	13.2 (1.5)	1.13 (0.25)	0.89 (<0.001)	14.2 (6.7)	10.1 (2.1)	1.51 (0.72)	0.085 (0.002)
aNDFom	49.6 (41.8)	43.5 (34.6)	2.65 (2.42)	0.13 (0.054)	53.0 (48.0)	44.9 (38.1)	2.90 (3.07)	0.079 (0.049)
ADFom	29.6 (26.8)	25.5 (22.2)	1.61 (1.55)	0.095 (0.060)	31.1 (29.7)	26.3 (23.8)	1.82 (1.89)	0.097 (0.055)
Lignin(sa)	5.9 (4.8)	4.7 (3.4)	0.33 (0.30)	0.017 (0.005)	9.6 (9.3)	4.9 (3.9)	1.07 (0.87)	0.013 (0.002)
Non-fibre CHO	16.4 (3.2)	16.7 (1.5)	1.45 (0.44)	0.88 (0.005)	21.5 (10.8)	15.2 (3.9)	2.18 (1.19)	0.073 (0.002)
EE	2.2 (0.8)	2.1 (0.5)	0.17 (0.08)	0.71 (0.012)	3.0 (1.7)	2.0 (0.6)	0.28 (0.17)	0.035 (0.002)
Total phenols	0.6 (0.5)	0.4 (0.2)	0.06 (0.06)	0.011 (0.007)	1.2 (1.2)	0.4 (0.3)	0.16 (0.13)	0.011 (0.001)

SEM, standard error of the means; n.d., not determined; –, not available.
[a] Non-fibre CHO, calculated as OM – aNDFom – CP – EE – total phenols.

Table 4
Consumption of individual plants and straw (g dry matter/kg live-weight$^{0.75}$ or in g/kg of total) when offered in the binary and multiple choice test to the choice group averaged across 7 day periods.

		Binary choice						Multiple choice	
		4 h			24 h			4 h	24 h
		Plant	Straw	Total	Straw	Total	Plant, g/kg of total	Test feed	Test feed g/kg of total
Experiment 1									
A. herba-alba		0.8^{bcB}	11.2^{A}	11.9^{c}	35.5^{a}	36.3^{c}	21^{c}	1.6^{cd}	33^{cd}
A. leucoclada		10.6^{a}	11.4	22.0^{a}	32.8^{b}	43.4^{a}	236^{a}	10.9^{a}	228^{a}
H. articulatum		1.2^{bB}	10.1^{A}	11.3^{c}	34.0^{ab}	35.2^{c}	34^{c}	0.1^{de}	2de
N. mucronata		0.2^{cB}	11.8^{A}	12.1^{c}	36.1^{a}	36.3^{c}	7^{c}	0.0^{e}	1^{e}
S. vermiculata		5.9^{aB}	12.3^{A}	18.3^{b}	34.1^{ab}	40.1^{b}	147^{b}	2.2^{c}	44^{c}
Straw		–	–	–	–	–	–	8.8^{b}	186^{b}
Feed:	SEM	0.84	1.20	1.08	1.47	1.63	18.80	0.38	7.12
	P-value	<0.001	0.17	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
Day:	SEM	0.84	1.34	1.15	1.52	1.41	19.56	0.40	7.71
	P-value	0.22	0.50	0.42	0.94	0.96	0.27	1.00	0.90
Feed × Day:	SEM	1.24	1.72	1.82	2.09	2.26	28.48	0.94	19.11
	P-value	0.97	0.68	0.95	0.83	0.99	0.95	0.18	0.076
Experiment 2									
A. leucoclada		3.4^{cdB}	18.7^{aA}	22.2^{c}	44.6^{c}	48.0^{c}	68^{cd}	4.4^{b}	55^{c}
B. pendula		24.4^{aA}	13.1^{bB}	37.4^{a}	36.3^{d}	60.7^{a}	388^{a}	29.9^{a}	385^{a}
C. sativa		5.8^{cB}	16.2^{aA}	22.0^{c}	47.4^{abc}	53.2^{b}	105^{c}	0.1^{c}	2^{d}
H. articulatum		0.5^{dB}	19.0^{aA}	19.4^{c}	48.0^{ab}	48.5^{c}	11^{d}	0.1^{c}	2^{d}
J. regia		13.9^{bB}	16.2^{aA}	30.1^{b}	45.3^{bc}	59.3^{a}	210^{b}	0.0^{c}	0^{d}
S. vermiculata		1.8^{cdB}	18.7^{aA}	20.5^{c}	49.0^{a}	50.8^{bc}	35^{cd}	0.0^{c}	0^{d}
Straw		–	–	–	–	–	–	6.9^{b}	104^{b}
Feed:	SEM	2.44	1.87	2.02	2.66	2.33	37.84	1.12	11.76
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Day:	SEM	2.49	1.90	2.08	2.68	2.78	38.49	1.12	11.76
	P-value	0.76	0.002	0.31	0.99	0.64	0.84	0.88	0.94
Feed × Day:	SEM	3.81	2.66	3.51	3.37	4.13	56.74	2.39	28.89
	P-value	1.00	1.00	1.00	0.92	1.00	0.99	1.00	1.00

–, not available; SEM, standard error of the means.
$^{a-e}$Within columns: LSmeans with different letters differ at P<0.05.
^{A,B}Within row: LSmeans of single test feed intake (4 h) with different letters differ at P<0.05.

In Exp. 1, feeding time during the first 30 min of the test period was longest (P<0.05) on *A. leucoclada* and *S. vermiculata* (≥10 min), but short (<5 min) on the other plants, with regard to time spent feeding on the single plants only (Table 5). In Exp. 2 animals fed longer (P<0.05) on the plants originating from temperate climate (*B. pendula*, *C. sativa*, and *J. regia*: >6 min) than on the Mediterranean plants (*A. leucoclada*, *H. articulatum* and *S. vermiculata*: <4 min). Total time spent feeding was higher in Exp. 2 for the binary choice of straw together with *B. pendula* and *J. regia* than with *H. articulatum*. However, in both experiments more time was spent on eating straw than test plants, except for *A. leucoclada* in Exp. 1 which was similarly long eaten than straw. The counts of visits for the single test plants and in total for the binary choice were lowest (P<0.05) for *N. mucronata* in Exp. 1, and for *H. articulatum* and *S. vermiculata* in Exp. 2. In both experiments they were higher for straw than for test plants, except for *A. leucoclada* and *S. vermiculata* in Exp. 1 and for *C. sativa* and *J. regia* in Exp. 2 (Table 5). There was a between-day variation in the counts of visits with regard to the single plants (P=0.003 in Exp. 1 and P=0.07 in Exp. 2; Table 5 and Fig. 2.1d, 2.2d). On day 1 of Exp. 1, the animals on average visited the test feeds more often than on days 4 and 7 (P<0.05; data not shown in figure).

3.3. Feeding pattern in the multiple choice situation

In both experiments, total test feed intakes during 4 h and 24 h were higher (P<0.05) in the multiple choice group than in the control group (Table 2). Straw intake was lower for the multiple choice group during 4 h (P=0.03 in Exp. 1 and P=0.001 in Exp. 2) but was not clearly different between the two treatment groups during 24 h (P=0.19 and P=0.08, for Exp. 1 and Exp. 2, respectively, Table 2). Straw intake during 4 h varied (P=0.003 and P<0.001, in Exp. 1 and Exp. 2, respectively) between days in both experiments, and there was an interaction (P=0.032) of day and group in Exp. 2 (Table 2). In detail, straw intake (4 h) on day 1 was lower (P<0.05) than on days 2, 3, 4 and day 7 in the control group in Exp. 2 (data not shown). In Exp. 2, total test feed intake during 4 h and 24 h (P<0.001 and P=0.03, respectively; Table 2), and straw intake during 24 h (P<0.001; Table 2) varied between days. In detail, straw intake (24 h) was lower (P<0.05) on day 1 than on day 7, and total test feed intake (4 h) was lower (P<0.05) on day 1 than on days 2–7 (data not shown). Intake of all nutrients and secondary compounds was higher (P<0.001) in the multiple choice group as compared to the control group in Exp. 1 with and without considering the amounts coming from the concentrate (Table 3). In Exp. 2, nutrient intakes from test feeds were also higher in the multiple choice group (P<0.05 relative to control in OM, CP, aNDFom, lignin(sa), non-fibre CHO, EE and TP; trend

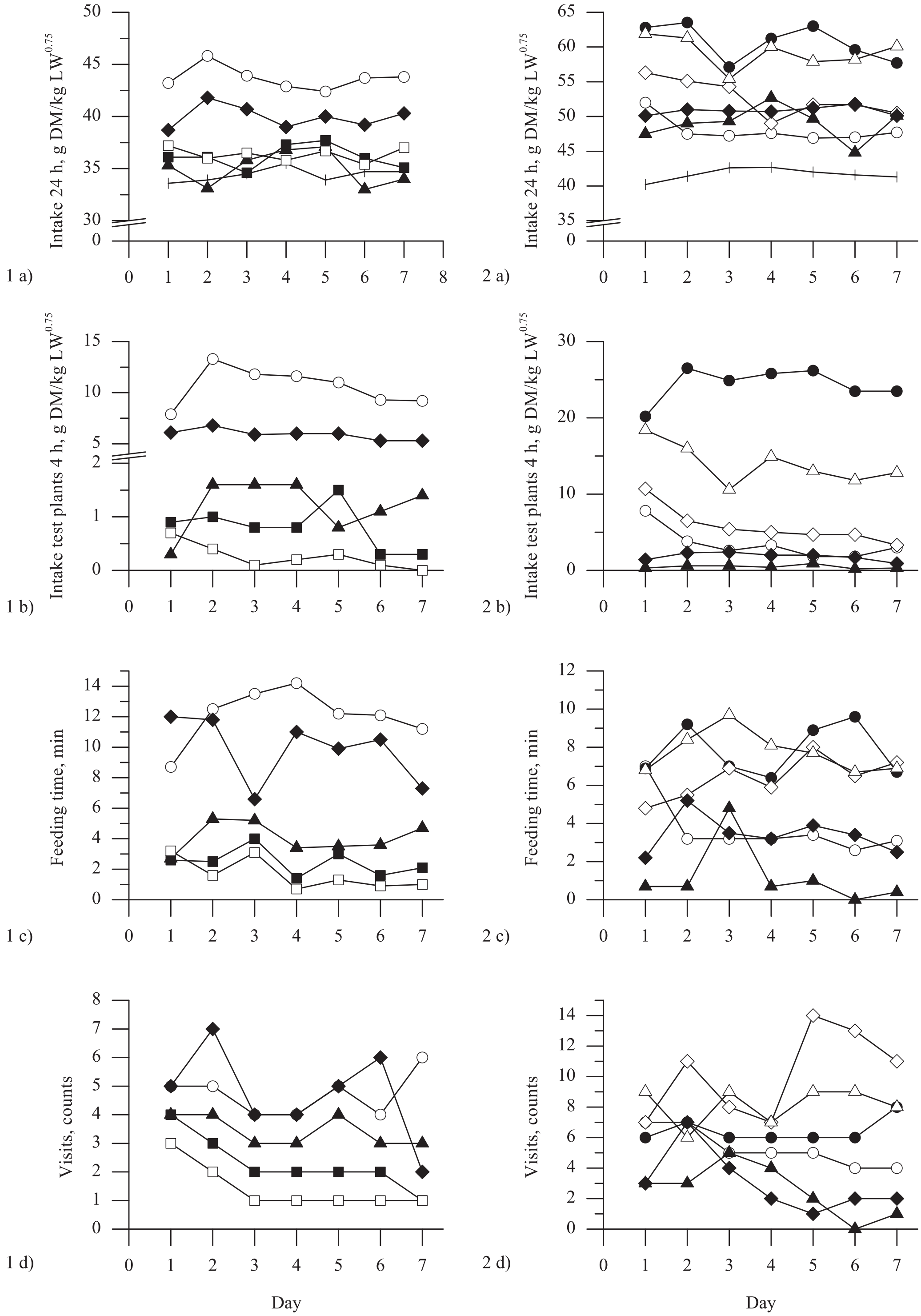

Fig. 2. Total daily test feed intake with the respective binary combinations (a; P-value and SEM given in Tables 2 and 4, for control and binary choice group, respectively), intake of single test plants during 4 h (b; P-value and SEM given in Table 4), feeding time (c; P-value and SEM given in Table 5) and counts of visits to individual test plants (d; P-value and SEM given in Table 5) during the binary choice test in Exp. 1 (1) and Exp. 2 (2); | control group, ■ *A. herba-alba*, ○ *A. leucoclada*, ● *B. pendula*, ◊ *C. sativa*, ▲ *H. articulatum*, △ *J. regia*, □ *N. mucronata*, ♦ *S. vermiculata*.

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Table 5
Feeding time (in min) and visits to the individual troughs (counts) of individual feeds during the binary or multiple choice test on average of the 7 day periods.

		Binary choice						Multiple choice	
		Feeding time			Visits			Feeding time	Visits
		Plants	Straw	Total	Plants	Straw	Total		
Experiment 1									
A. herba-alba		2.5^{bcB}	19.8^{aA}	22.3	2.5^{bB}	4.6^{A}	7.0^{ab}	1.8^{bc}	2.3^{b}
A. leucoclada		12.0^{a}	12.7^{c}	24.8	4.8^{a}	3.9	8.7^{ab}	12.3^{a}	7.2^{a}
H. articulatum		4.1^{bB}	15.6^{abcA}	19.6	3.2^{abB}	5.3^{A}	8.5^{ab}	0.1^{c}	0.7^{c}
N. mucronata		1.7^{cB}	18.5^{abA}	20.3	1.6^{cB}	5.2^{A}	6.8^{b}	0.0^{c}	0.3^{c}
S. vermiculata		9.9^{aB}	14.7^{bcA}	24.5	4.6^{a}	4.3	8.9^{a}	4.1^{b}	3.8^{b}
Straw		–	–		–	–		9.8^{a}	6.5^{a}
Feed:	SEM	1.10	2.29	2.52	0.52	0.46	1.01	0.66	0.81
	P-value	<0.001	<0.001	0.027	<0.001	0.10	0.008	<0.001	<0.001
Day:	SEM	1.20	0.29	2.67	0.56	0.53	1.07	0.71	0.83
	P-value	0.18	0.34	0.48	0.003	0.030	<0.001	1.00	0.013
Feed × Day:	SEM	2.16	0.48	4.24	1.03	1.14	1.65	1.83	1.34
	P-value	0.78	0.74	0.91	0.67	0.66	0.19	0.43	0.088
Experiment 2									
A. leucoclada		3.7^{bB}	16.7^{A}	20.2^{ab}	5.2^{bB}	9.9^{aA}	15.2^{bcd}	2.5^{b}	6.1^{b}
B. pendula		7.8^{aB}	14.5^{A}	22.1^{a}	6.7^{abB}	8.9^{aA}	15.6^{bc}	12.8^{a}	15.0^{a}
C. sativa		6.4^{aB}	15.0^{A}	21.1^{ab}	10.2^{a}	11.8^{a}	22.0^{a}	0.0^{b}	0.0^{c}
H. articulatum		1.2^{cB}	17.2^{A}	18.2^{b}	2.6^{cB}	8.5^{abA}	11.1^{cd}	0.0^{b}	0.2^{c}
J. regia		7.7^{aB}	14.3^{A}	21.6^{a}	8.1^{a}	9.7^{a}	17.8^{ab}	0.0^{b}	0.0^{c}
S. vermiculata		3.4^{bcB}	15.8^{A}	19.0^{ab}	3.0^{cB}	6.9^{bA}	9.9^{d}	0.0^{b}	0.0^{c}
Straw		–	–		–	–		11.1^{a}	10.7^{a}
Feed:	SEM	1.34	1.38	2.01	1.50	1.20	2.59	1.02	0.87
	P-value	<0.001	0.047	0.005	<0.001	<0.001	<0.001	<0.001	<0.001
Day:	SEM	1.35	1.39	2.04	1.53	1.24	2.64	0.95	0.84
	P-value	0.69	0.59	0.72	0.073	0.32	0.86	0.78	0.76
Feed × Day:	SEM	2.02	2.33	2.84	2.26	2.06	4.08	2.80	2.29
	P-value	0.94	0.54	0.80	0.14	0.94	0.81	0.99	0.018

–, not available; SEM, standard error of the means.
[a–c]Within columns and experiments: LSmeans with different letters differ at P<0.05.
[A,B]Within row: LSmeans of feeding time or visits of single test feed with different letters differ at P<0.05.

(P<0.1) for ADFom). Nutrient intakes in Exp. 2 were higher (P<0.05 relative to control in OM, lignin(sa), EE and TP) or showed a trend (P<0.1 relative to control in CP, aNDFom, ADFom and non-fibre CHO) when including the nutrients provided by the concentrate. The level of significance was lower for nutrient intakes when the nutrients provided by the concentrate were considered.

In Exp. 1, the most consumed (P<0.05) test feed during 4 h test period was *A. leucoclada*, followed by straw (about 230 and 190 g/kg of total test feed intake, respectively; Table 4). In Exp. 2, *B. pendula* was the most preferred (P<0.05) test feed and intake amounted to almost 400 g/kg of total test feed intake followed by straw (104 g/kg) and *A. leucoclada* (55 g/kg). The other plants were almost neglected (<2.5 g DM/kg live-weight$^{0.75}$; <3 g/kg of test feed intake).

In Exp. 1, feeding time during the first 30 min of the test period was longest (P<0.05) for *A. leucoclada* and straw (12 and 10 min, respectively; Table 5). Troughs filled with these two feeds were also most frequently (P<0.05) visited (*A. leucoclada* and straw both on average 7 visits). *H. articulatum* and *N. mucronata* were almost completely neglected (feeding time <1 min and one or no visit). In Exp. 2, the longest (P<0.05) feeding times (13 and 11 min) and the highest (P<0.05) number of visits (15 and 11) were recorded for *B. pendula* and straw, respectively. *C. sativa*, *H. articulatum*, *J. regia*, and *S. vermiculata* were almost neglected. The number of visits on the different plants varied (P=0.01) between days in Exp. 1 (Table 5 and Fig. 3.1d), and there was an interaction (P=0.02) of test feed and day in Exp. 2 (Table 5 and Fig. 3.2d). In Exp. 1 on average the number of visits of the test feeds were higher (P<0.05) on day 1 (4.6) than on day 4 (4.3) and 6 (2.9; data not shown). In Exp. 2, the number of visits of the trough filled with *A. leucoclada* was higher (P<0.05) on day 4 than on day 3 (Fig. 3.2d).

4. Discussion

4.1. Woody test plants: nutritional value and preference under different choice situations

In the choice situations assessed in this study, the animals showed different preferences for the tested woody plants; however, the animals selected the preferred plants not exclusively and still included minor proportions of the other test feeds. This was similar in a study by Rutter (2006) where the animals were given the choice among more than one feed alternative (here clover and ryegrass). Illius et al. (1999) offered choices between sward patches of different temperate grasses to goats

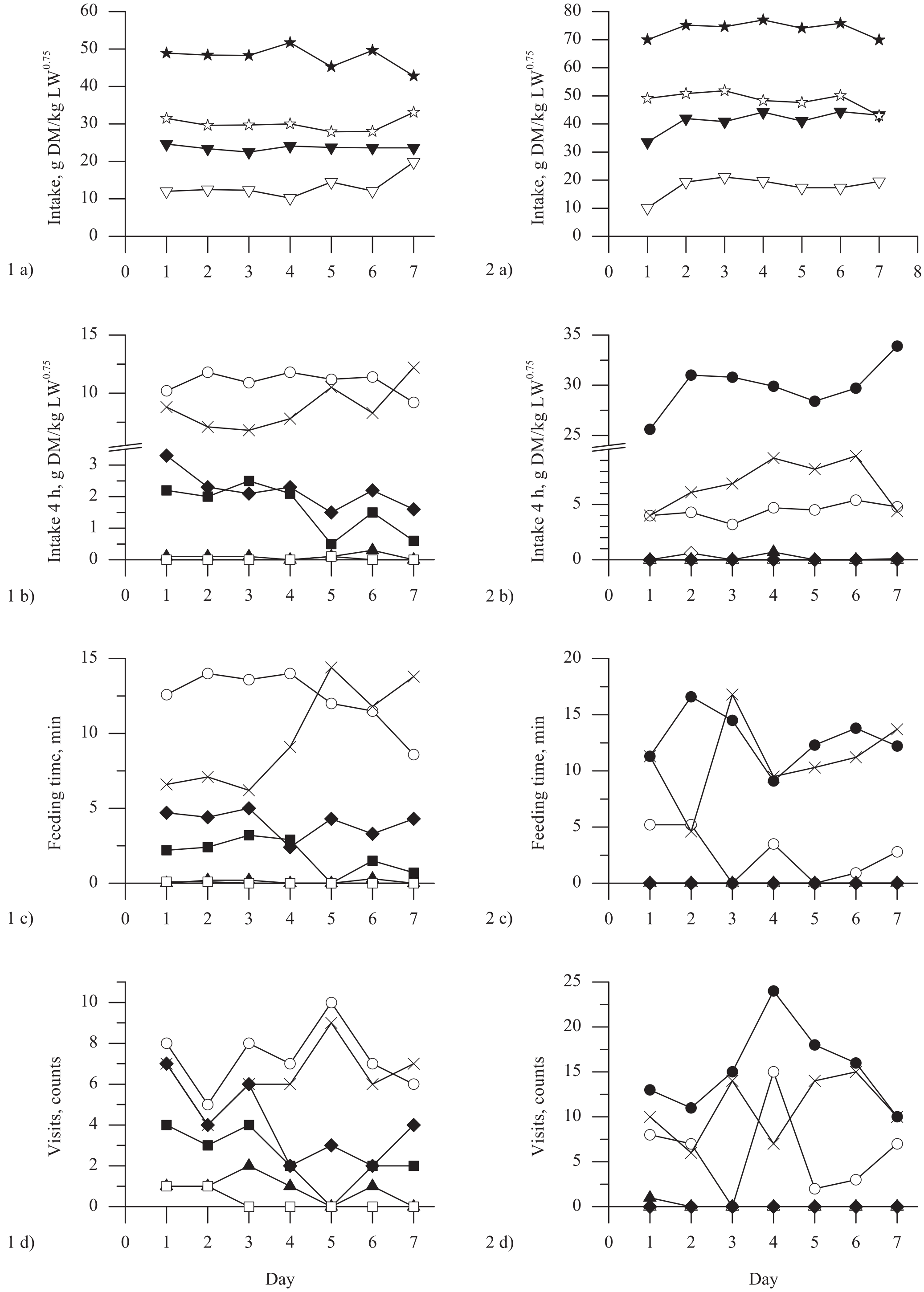

Fig. 3. Total test feed intake (a; SEM given in Table 2) within 4 h (▾ choice and ▿ control group) and 24 h (⋆ choice and ☆ control group), intake of single test feeds during 4 h (b; P-value and SEM given in Table 4), feeding time (c; P-value and SEM given in Table 5) and counts of visits to individual test feeds (d; P-value and SEM given in Table 5) during the multiple choice test in Exp. 1 (1) and Exp. 2 (2); ■ *A. herba-alba*, ○ *A. leucoclada*, ● *B. pendula*, ◊ *C. sativa*, ▲ *H. articulatum*, △ *J. regia*, □ *N. mucronata*, ♦ *S. vermiculata*, × straw.

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and expected feeds with high intake rates to be highly preferred, but still there were only partial preferences for the grasses with high intake rates. They explained this by the inability of the animals to sufficiently discriminate between the feeds. Another reason for partial preferences could be sampling behaviour with the purpose of continuously re-assessing the nutritional characteristics of all feeds by ingesting small amounts which had been concluded by Ginane et al. (2002) from an experiment where heifers were offered a choice among two hays of different quality showed high numbers of switching between the two hays and short eating events for the hay of lower quality. It seems also likely that the partial preferences expressed in the binary and multiple choice tests were an expression of sampling behaviour. The partial preferences found in the binary choice tests are probably also resulting from the fact that the test plants were the only available alternative to straw. In the present study, the proportional intake of the most preferred plants (*A. leucoclada* in Exp. 1 and *B. pendula* in Exp. 2) was similar in the binary and multiple choice tests (about 230 g/kg and 390 g/kg of total daily test feed intake, respectively). However, when offered as a multiple choice, the proportion of the less preferred plants and straw in comparison to the preferred plants decreased.

It seems that the feeding value had only a limited influence on choice. Even though in Exp. 1 *A. leucoclada* had the highest forage quality of all Mediterranean woody plants investigated, the most consumed plant in Exp. 2, *B. pendula*, was rich in fibre and lignin(sa), even when compared to low-quality straw, and richer in TP and TT compared to the Mediterranean plants. Therefore, *B. pendula* had a clearly lower feeding value than *A. leucoclada*, which was consumed to a much lesser extent. Owing to the extra concentrate and the comparably low milk yield, covering energy requirements by the forages seems not to have been of priority for the sheep in both experiments. *J. regia* was the second most consumed test feed in the binary choice test of Exp. 2, where the low-CP straw was the only alternative, but was almost neglected in the multiple choice test.

4.2. Choice vs. no-choice situations with low-quality forages

In cases where animals are confronted with low quality feeds, giving the choice may be advantageous as nutrients could be selected according to the animal's individual nutritional requirements (Provenza et al., 2003). Thus the individual animal may regulate the intake of nutrients and toxins (Rogosic et al., 2012) which would help preventing an excessive intake of noxious substances (Manteca et al., 2008). There were, however, no indications for an attempt of the animals in the present study to avoid excessive concentrations of substances by combining complementary test plants. As expected, the animals of the present study increased their intake in a multiple choice situation where different woody plants were offered as compared to a no-choice situation with only barley straw. The higher test feed intake came along with a higher supply with nutrients and, likely, energy. Seen over 4 h and 24 h, the higher feed and nutrient intake resulted mostly from the extra test plant intake. Even though straw intake was reduced in the test period, this was compensated by a higher straw intake during the rest of the day.

The finding of a higher feed intake in a choice *vs.* a no-choice situation is consistent with previous results. Distel et al. (2007) found a tendency for an increased intake of lambs when hay enriched with different flavours was offered simultaneously in a choice situation as compared to a control group receiving only non-flavoured hay as single feed. Provenza et al. (1996) described that lambs enhanced their intake when several feeds were offered simultaneously as compared to offering only one feed, no matter whether the feeds on offer had similar or different quality, or differed in flavour. Ginane et al. (2002) reported a higher daily DM intake when a choice among two hays of different or similar quality was offered than when one of these hay types was offered alone and explained this by an increased motivation to eat when a diverse offer is available. Even though high quality feeds are preferred by the animals in a choice situation, feeds with lower quality might help to motivate intake simply by increasing the diversity on offer (Ginane et al., 2002), because monotonous diets might lead earlier to a sensory-specific satiety (Rolls, 1986) thus decreasing the motivation to eat due to the lack of feed alternatives (Villalba et al., 2010). When testing different Mediterranean woody forages offered for 6 h/d by varying the number of feeds on offer between each 5-day period, intake increased or decreased along with the number of feeds (Rogosic et al., 2006, 2007) which was explained by the changing biochemical diversity. Accordingly, the increase in feed intake in the present experiments seemed to be higher in the multiple choice situation than in the binary choice situation.

5. Conclusions

In both experiments, a higher diversity in the low quality forages offered either in a binary or a multiple choice resulted in higher intakes of total test feeds and of several nutrients compared to a no-choice situation (only barley straw). This experimentally confirmed both hypotheses tested (*i.e.*, higher feed and nutrient intake when a choice is offered compared to a no-choice situation), and this in two different situations with different animal genotypes and partially differing low quality feeds. From the present study, also some conclusions can be drawn about palatability and feeding value for sheep of rarely investigated woody plants. Especially the leaves of *B. pendula* and the leaves with twigs of *A. leucoclada* and, to a much lesser extent, of *S. vermiculata* were promising to be used as dried forage supplements. As typically mixed flocks with sheep and goats are kept on rangelands in the Mediterranean area further research is needed to investigate possible benefits of the tested woody plants when offered as fresh feeds and to other ruminant species. In addition, the efficiency of offering a higher feed diversity should be tested with a control feed other than that offered in the basal diet.

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